Supplementary Table 1. Characteristics of all human IGF-PCa studies used for circulatory and genetic meta-analyses, stratified by study design and ordered by year of publication.

Author (year)	Study Name	Study Location	Assay	Control Type	Control Source	Mean age at diagnosis (years)	Average age of cases (years)	Average age of controls (years)	Median time between (years)	Ethnicity	IGF-I	IGF-II	IGFBP-1	IGFBP-2	IGFBP-3	Genetic	Advanced PCa risk	PSA Screened	Data used	Data adjusted?	Overall RoB
Prospective studies																					
Chan (1998)	Physicians' Health Study	USA	ELISA	Mixed	PBC		M 5yr	60.3	7	Not Stated	√	√			√				Quantiles[1]	√	Moderate
Harman (2000)	Baltimore Longitudinal Study of Aging	USA	RIA	Healthy	PBC	74	64.8	65.7	12.8	Multi-ethnic	√	√			√				Quantiles	√	Moderate
Stattin (2000)	NSHDS	Sweden	IRMA	Mixed	PBC	63	59.7	59.6	3.3	Caucasian	√		√	√	√				Quantiles	√	Moderate
Stattin (2001)	NSHDS	Sweden	IRMA	Mixed	PBC		58.4	59.5	3.9	Caucasian	√				√				None: RCD	√	Moderate
Chan (2002)	Physicians' Health Study	USA	ELISA	Healthy	PBC		M 1yr	M 1yr	9	Not Stated	√				√				None: RCD	√	Moderate
Li (2003)		USA	ELISA	Mixed	S		61.3	62.8	2	Multi-ethnic	√				√		√		Quantiles	√	Moderate
Woodson (2003)	ATBC Trial	Finland	ELISA	Healthy	TC	68.6	59	56.4	9.6	Not Stated	√				√				Quantiles	√	Moderate
Janssen (2004)	ERSPC	Netherlands	IRMA	Healthy	TC	66.4	62.3	62.3	4	Not Stated	√				√			√	None: RCD	√	Moderate
Stattin (2004)	NSHDS	Sweden	IRMA	Mixed	PBC	63.6	59.9	59.9	3.7	Caucasian	√				√				Quantiles	√	Moderate
Chen (2005)	Cardiovascular Health Study	USA	IRMA	Mixed	PBC		72.3	72.3	3.4	Multi-ethnic	√				√		√		Quantiles	√	Moderate
Meyer (2005)	SUVIMAX Trial	France	CLA	Healthy	TC		55.2	55.2	5+	Not Stated	√	√		√	√				Quantiles	√	Moderate
Platz (2005)	Health Professionals Follow Up Study	USA	ELISA	Healthy	PBC	68.6	68.6	M 1yr	2.2	Not Stated	√				√		√		Quantiles	√	Moderate
Morris (2006)	BUPA study	UK	ELISA	Healthy	PBC		52.4	52.4	9.7	Not Stated	√	√			√				Means		Moderate
Severi (2006)	Melbourne collaborative cohort	Australia	ELISA	Mixed	PBC	67			3+	Multi-ethnic	√				√				Excluded		Critical
Allen (2007)	EPIC	Europe	ELISA	Mixed	PBC	65			3.4	Multi-ethnic	√				√		√		Quantiles	√	Moderate
Li (2007)	Physicians' Health Study	USA	ELISA	Mixed	PBC	69.4	58.9	59	11	Caucasian	√				√				Means	√	Moderate
Weiss (2007)	PLCO screening trial	USA	ELISA	Healthy	PBC		>55	>55	1+	White	√				√		√	√	Quantiles	√	Moderate
Mikami (2009)	Japan Collaborative Cohort Study	Japan	IRMA	Mixed	PBC		69.3	69.1	5.3	Asian	√				√				Means	√	Moderate

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Gu (2010)	BPC3		ELISA	Mixed	PBC		62.3	63.2	4.5	Caucasian	√				√				None: RCD	√	Moderate
Nimptsch (2011)	Health professionals follow up study	USA	ELISA	Mixed	PBC	65	M 1yr	M 1yr	6	Multi-ethnic	√				√		√		Continuous	√	Moderate
Price (2012)	EPIC	Europe	ELISA	Mixed	PBC		60.12	60.1	3.4	Not Stated	√						√		Quantiles	√	Moderate
Tsilidis (2012)	BPC3		ELISA	Mixed	PBC		62.9	60.2	4.9	Caucasian	√				√				None: RCD	√	Moderate
Tsilidis (2013)	BPC3		ELISA	Mixed	PBC		66.2	65.9	2.1	Caucasian	√				√	√			None: RCD	√	Moderate
Muhlbradt (2014)	Physicians' Health Study	USA	ELISA	Mixed	PBC	70		62	8	Caucasian	√								Quantiles[2]	√	Moderate
Retrospective studies																					
Cohen (1993)		USA	RIA	Healthy	P	Not stated				Not Stated	√	√		√	√				Means	√	Moderate
Kanety (1993)		Israel	RIA	Healthy	NS		65.8	56.4	23m	Not Stated				√	√				Excluded		Critical
Ho (1997)		Australia	RIA	Mixed	H		74.4	68.6		Not Stated	√	√		√	√				Excluded		Critical
Mantzoros (1997)		Athens, Greece	RIA	Healthy	P		71.9	71.8		Not Stated	√								Continuous	√	Moderate
Wolk (1998)		Sweden	IRMA	Healthy	P		70	M 10yrs		Not Stated	√				√		√		Quantiles, Continuous	√	Serious
Schaefer (1998)		USA	RIA	Healthy	P	71			1+	Not Stated	√								Rowlands data		Unclear
Cutting (1999)		UK	IRMA	Healthy	H		73.2	67.4		Not Stated	√								Excluded		Critical
Djavan (1999)		Austria	IRMA	Mixed	H		65.7	67.7		White	√								Means		Serious
Signorello (1999)		Sweden	IRMA	Mixed	P		69.9	70.9		Not Stated	√		√		√				Means	√	Moderate
Hill (2000)		Czech Republic	IRMA	BPH	H		77.1	72.2		Not Stated	√								Means		Serious
Koliakos (2000)		Greece	IRMA	BPH	H		67	69		Not Stated	√								Means		Serious
Baffa (2000)		USA	ELISA	Healthy	NS	Not stated				Not Stated	√								Means	√	Moderate
Finne (2000)	Finnish Prostate Cancer Screening Trial	Finland	ELISA	Mixed	PBC		62	62.6	2-18m	Not Stated	√				√			√	Continuous	√	Moderate
Kurek (2000)		Germany	CLA	Healthy	NS		66.2	64.5		Not Stated	√								Means	√	Moderate

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Lacey (2001)		USA	ELISA	Healthy	PBC	70.6				White	√				√				Means	√	Moderate
Chokkalingham (2001)		China	ELISA	Mixed	P		71.9	72		Asian	√	√	√		√				Quantiles	√	Moderate
Khosravi (2001)		Canada	ELISA	BPH	NS		64.8	65.6		Not Stated	√				√				Means		Serious
Li (2001)		China	RIA	Mixed	H		74.7	74.8		Asian	√								Quantiles		Moderate
Perk (2001)		Turkey	IRMA	Healthy	H		62.3	36		Not Stated	√								Excluded		Critical
Ismail (2002)		Montreal, Canada	ELISA	Mixed	H		64.6	64.5		Not stated	√				√				Means	√	Serious
Shariat (2002)		USA	ELISA	Healthy	H	63	29	44		Not Stated	√			√	√				Rowlands data		Unclear
Peng** (2002)		China	IRMA	Mixed			74.8	64.8		Not Stated	√								Excluded		Critical
Kaaks (2003)	NSHDS	Sweden	RIA	Mixed	PBC				1m-10yrs	Caucasian	√								None: RCD		Moderate
Miyata (2003)		Nagasaki University, Japan	IRMA	BPH	H		70.9	69.8		Asian	√				√		√		Means		Serious
Scorilas (2003)	Padova	Italy	ELISA	BPH	H		68	65		Not Stated	√								Means		Serious
Aksoy (2004)		Turkey	IRMA	BPH	NS		53-85	51-79		Not Stated	√				√		√		Means		Serious
Oliver (2004)	ProtecT	UK	ELISA	Mixed	PBC		62.2	62.2		Caucasian	√	√		√	√		√	√	Quantiles	√	Moderate
Trapeznikova (2004)		Russia	ELISA	BPH	NS		66.6	60.3		Not Stated	√	√							Excluded		Critical
Lopez (2004)		Malaysia	ELISA	Healthy	NS		69.7	57.2		Not Stated	√				√				Excluded		Critical
Kehinde (2005)		Kuwait/Oman	IFMA	Healthy	P	69.7	15-90	15-90		Caucasian	√				√				Excluded		Critical
Marszalek (2005)		Austria	IRMA	Mixed	NS	66.7	67	69		Not Stated	√								Means		Moderate
Nam (2005)	University Health Network	Canada	ELISA	Mixed	TC		66.6	65.5		Multi-ethnic	√				√				Means		Serious
Trojan (2006)			ELISA	BPH	H		62.8	66.8		Not Stated		√							Means		Serious
Hernandez (2007)		USA	Other	Healthy	H		65.86	68.85		Black	√				√	√	√		Quantiles	√	Moderate

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Zhigang (2007)		China	ELISA	Mixed	H		65.5	65.1		Asian	√				√				Means		Moderate
Borugian (2008)	Prospective Multiethnic Study	Hawaii, USA, Canada	ELISA	Mixed	P		69.1	68.9	1+	Multi-ethnic	√				√				Quantiles	√	Moderate
Hong (2008)		Korea	IRMA	Healthy	H		65	64		Asian	√				√				Means		Serious
Sciarra (2008)		Italy	ELISA	BPH	H		67.24	67.06		Not Stated	√								Means		Serious
Jeong*** (2009)		Korea	ELISA	Healthy	P		63.5	63.1		Asian	√				√		√		Quantiles	√	Moderate
Pina (2009)		Portugal	ELISA	BPH	NS		69	67		Not Stated	√								Means		Serious
Johansson (2009)	CAPS Study	Sweden		Healthy	P		M 5yr	M 5yr		Caucasian					√	√			Means	√	Moderate
Gill (2010)	MEC	USA	ELISA	Mixed	P		68.9	68.7		Multi-ethnic	√	√	√		√		√		Quantiles	√	Moderate
Kim (2010)		Korea	ELISA	Mixed	H		Not stated			Not Stated				√	√				Rowlands data	√	Moderate
Park*** (2010)		Korea	ELISA	Healthy	H		64.7	63.5		Asian					√	√			Means	√	Moderate
Tajtakova (2010)		Slovakia	RIA	Healthy	NS		65.5	60.7		Not Stated	√				√				Means		Serious
Campa (2011)	EPIC	Europe	ELISA	Mixed	PBC	60.4	60.4	60.5	1*	Caucasian	√				√				None: RCD		Moderate
Darago (2011)		Poland	CLA	BPH	H		70.2	70.1		Not Stated	√				√				Means		Serious
Safarinejad (2011)		Iran	ELISA	Healthy	H		63.6	62.5		Caucasian	√				√	√			Means	√	Moderate
Rowlands (2012)	ProtecT	UK	RIA	Mixed	PBC		61.9	61.7		White	√	√		√	√			√	Continuous	√	Moderate
Neuhouser (2013)	PCPT	USA	ELISA	Mixed	TC		63.6	63.6		White	√	√		√	√			√	Means	√	Moderate
Iltaf (2013)		Karachi	ELISA	Healthy	P			50+		Asian	√								Excluded		Critical
Genetic data only																					
Ho (2003)		USA		Mixed	H	63				Multi-ethnic						√			Categorical	√	Low

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Wang (2003)		Japan		Healthy	P					Multi-ethnic						√			Categorical	√	Low
Li (2004)		USA		Mixed						Multi-ethnic						√			Categorical	√	Low
Friedrichsen (2005)	Seattle-Puget Sound Registry SEER	USA		Mixed	P					Multi-ethnic						√			Categorical	√	Low
Neuhausen (2005)		University of Utah, USA		Mixed	P	63				Multi-ethnic						√			Categorical	√	Unclear
Schildkraut (2005)		USA		Mixed	P	62.7				Multi-ethnic						√			Categorical	√	Unclear
Tsuchiya (2005)		Japan		Healthy	H					Caucasian						√			Categorical	√	Low
Chen (2006)	Cardiovascular Health Study	USA		Mixed	PBC					Multi-ethnic						√			Categorical	√	Low
Cheng (2006)	MEC	USA		Mixed	PBC					Caucasian						√			Categorical	√	Unclear
Cheng (2006)	MEC	USA		Mixed	PBC	68.3				African-American						√			Categorical		Unclear
Hoyo (2007)		USA		Mixed	H					Multi-ethnic						√			Categorical	√	Low
Johansson (2007)	CAPS Study	Sweden		Healthy	P					Caucasian						√			Categorical		Unclear
Sarma (2008)	Flint Men's Health Study	USA		Mixed	P					Asian						√			Categorical	√	Critical
Schumacher (2010)	BPC3	Mixed	ELISA	Mixed	PBC	68				Asian						√			Categorical	√	Low

BPH: Benign prostatic hyperplasia; M: Matched; H: Hospital; P: Population; PBC: Population-based cohort; RCD: Repeated cohort data; S: Sibling; TC: Trial cohort.

ATBC: Alpha-Tocopherol, Beta-Carotene Cancer Prevention; BPC3: NCI Breast and prostate cancer consortium; CAPS: Cancer of the Prostate in Sweden; EPIC: European Prospective Investigation into Cancer and Nutrition; MEC: Multi-ethnic cohort; NSHDS; Northern Sweden Health and Disease Cohort Study; PCPT: Prostate cancer prevention trial; PLCO: Prostate, Lung, Colorectal and Ovarian.

**Minimum number of years between sample collection and diagnosis.*

***Unclear if retrospective or prospective study; therefore, grouped as retrospective*

****Unclear whether PSA-screened or not.*

[1]IGF-I only

[2]RFPC study only

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Supplementary Table 2. List of polymorphisms presented in each genetic paper (highlighted columns represent those polymorphisms with sufficient data to be analysed.

Author (year)	IGF-1 (CA)n*	IGF-1 hCV2801089	IGF-1 hCV2801106	IGF-1 hCV2801114	IGF-1 hCV3061153	IGF1 rs10735380	IGF-1 rs1106381	IGF-1 rs1457601	IGF1 rs1520220	IGF-1 rs1520220	IGF-1 rs2033178	IGF-1 rs2072952	IGF-1 rs2288377	IGF-1 rs2946834	IGF-1 rs4764695	IGF-1 rs4764876	IGF-1 rs5742639	IGF-1 rs5742657	IGF-1 rs5742723	IGF-1 rs6218	IGF-1 rs6220	IGF-1 rs7136446	IGF-1 rs7965399	IGF-1 rs7978742	IGF-1R (AGG)n	IGF-1R deletion	IGF2-Msp1 +3580	IGFALS rs1178436	IGFALS rs11865665	IGFALS rs17559	IGFALS rs344352	IGFBP1 rs1065780	IGFBP1 rs1995051	IGFBP1 rs3763497	IGFBP1 rs4988515	IGFBP1 rs9658194	IGFBP3 -202A/C*	IGFBP3 rs2132570	IGFBP3 rs2132571	IGFBP3 rs2270628	IGFBP3 rs2453839	IGFBP3 rs2471551	IGFBP3 rs2854742	IGFBP3 rs2854744	IGFBP3 rs2854746	IGFBP3 rs2960436	IGFBP3 rs3110697	IGFBP3 rs6670
Chen (2006)	√																								√	√										√										
Cheng (2006)																																				√										
Cheng (2006)		√	√	√	√		√	√		√		√	√	√		√	√	√	√	√			√	√																					
Friedrichsen (2005)	√																																								√				
Hernandez (2007)	√																						√													√									
Ho (2003)																										√																			
Hoyo (2007)**	√																																			√										
Johansson (2009)																															√	√	√	√	√			√		√	√		√				√
Johansson (2007)											√										√	√																							
Li (2004)**	√																					√														√									

Neuhausen (2005)**	√																																															
Park (2010)																																					√											
Safarinejad (2011)																																					√											
Schildkraut (2005)	√																																				√						√					
Schumacher (2010)															√																																	
Tsilidis (2013)						√			√																			√	√	√	√							√		√				√	√	√	√	
Tsuchiya (2005)	√																																															
Wang (2003)																																					√											

Only IGF-1(CA)$_n$ and IGFBP-3 -202A/C were meta-analysed.

***Could not be used in meta-analysis as data was un-combinable (Li (2004) had combinable IGFBP-3 data, but un-combinable IGF-I data)*

Supplementary Table 3: Studies investigating tissue expression of IGF system and prostate cancer included as supporting evidence.

Study	Year	Experiments	Samples	Statistical analysis	Results
IGF-I					
Mita	2000	**qPCR** analysis of mRNA of IGF system	24 prostatectomy specimens after neoadjuvant hormone therapy No controls included.	Mann-Whitney U-test	IGF-I mRNA was lower in locally advanced prostate cancer than in early stage (p=0.038), but was not related to LN mets, histologic differentiation or serum PSA level.
Cardillo	2003	**IHC**, **ISH** and **qPCR** of IGF-I, IGF-II and IGF-IR protein and mRNA expression in PCa, PIN and NAP	Prostatectomy specimens Control: PIN and NAP	Mean +/- SEM, Chi-square, Student's paired t-test, Mann-Whitney U-test, Spearman rank correlation and linear regression , ANOVA	IGF-I protein increased from NAP tissue to PIN, to PCa tissue in the epithelial (p<0.0001) and stromal (p<0.0146). No correlation between IGF-I (protein and mRNA) and Gleason histological score or TNM stage.
Soulitzis	2006	**qPCR** to determine mRNA expression levels of IGF-I in tissue specimens from patients with PCa, or BPH, and normal prostate samples obtained post-mortem from young individuals	Patients with PCa (n=42) BPH: (n=42) Controls: young individuals (n=10)	Chi-squared test and Fisher's exact test	PCa patients with low Gleason score (<7) have increased IGF-I (p=0.031) mRNA levels. IGF-I levels are also elevated in tumors with TNM stages T1-T2 (p=0.03).
Massoner	2011	**IHC** and **qPCR** of IGF axis (IGF-I, IGF-II, IGFBP 1-6 and insulin receptor) in microdisseced tissue specimens of local PCa	Set 1: 20 local PCa specimens assigned according to their Gleason score used for laser microdissection Set 2: 10 local PCa specimens Set 3: 22 samples for IHC together with 20 samples from set 1 Benign tissues (from prostatectomy specimens)	Spearman's p test for correlation, Mann-Whitney U-test and student's t-test	IGF-I mRNA expression was decreased in prostate cancer compared with benign prostate areas. IGF-I mRNA was decreased in high-grade (GSC 8–10) compared with low- grade (GSC 5– 6) cancer. IGF-I protein levels determined by IHC do not reflect mRNA expression levels in PCa.
Savvani	2013	**IHC** of IGF-IEc expression in prostate cancer specimens	83 prostatectomy specimens No controls	Shapiro-Wilk test, Student's t-test, ANOVA and spearman correlation coefficient	Mean IGF-1Ec expression was lower in localized (stage ≤IIb) PCa compared to locally advanced tumours (stage ≥III) (p=0.004). Weak positive correlation was observed between IGF-IEc expression and Gleason score (p=0.02). No association between IGF-IEc expression and age (p=0.81), PIN (p=0.153), positive surgical margins (p=0.95), vascular invasion (p=0.347), perineural invasion (p=0.185) and tumour extent inside the prostate gland (p=0.18).

Study	Year	Experiments	Samples	Statistical analysis	Results
IGF-II					
Tennant	1996	**IHC** and **ISH** to compare the expression of IGF-IR and IGF-II in benign epithelium, HG-PIN and prostate adenocarcinoma	32 prostatectomy specimens	ANOVA, Fisher's exact test and paired t-tests	IGF-II mRNA was increased by 30% in adenocarcinoma compared to benign epithelium ($p<0.03$) but not IGF-II protein.
Mita	2000	**qPCR** analysis of mRNA of IGF system	24 prostatectomy specimens after neoadjuvant hormone therapy; No controls included.	Mann-Whitney U-test	IGF-II mRNA was associated with pathologic stage ($p=0.003$), LN mets ($p=0.0007$), histologic differentiation ($p=0.003$) and serum PSA level ($p=0.04$) after hormone therapy.
Cardillo	2003	**IHC**, **ISH** and **qPCR** of IGF-I, IGF-II and IGF-IR protein and mRNA expression in PCa, PIN and NAP	Prostatectomy specimens Control: PIN and NAP	Mean +/- SEM, Chi-square, Student's paired t-test, Mann-Whitney U-test, Spearman rank correlation and linear regression and ANOVA	IGF-II protein increased as the prostate tissue progressed from NAP, to PIN and PCa in both epithelium ($p<0.0001$) and stroma ($p=0.033$). IGF-II mRNA increased as the prostate tissue progressed from NAP, to PIN and PCa in the epithelium ($p<0.0001$) and in the stroma ($p=0.04$). No correlation was found between TNM stage and IGF-II expression. High Gleason score tumors (8,9) expressed IGF-II mRNA and protein more strongly than lower Gleason score tumors, in the epithelium (IGF-II protein 2.85±0.14 vs 1.90±0.28, $p=0.012$; IGF-II mRNA 2.62±0.10 vs 1.96±0.22, $p=0.03$) and in the stroma (IGF-II protein 2.71±0.18 vs 2.06±0.22, $p = 0.048$; IGF-II mRNA 2.23±0.1 vs 1.69±0.2, $p=0.05$).
Massoner	2011	**IHC** and **qPCR** of IGF axis (IGF-I, IGF-II, IGFBP 1-6 and insulin receptor) in microdisseced tissue specimens of local PCa	Set 1: 20 local PCa specimens assigned according to their Gleason score used for laser microdissection; Set 2: 10 local PCa specimens; Set 3: 22 samples for IHC together with 20 samples from set 1 Control: Benign tissues (from prostatectomy specimens)	Spearman's p test for correlation, Mann-Whitney U-test and student's t-test	IGF-II mRNA expression was decreased in PCa compared with benign prostate areas. IGF-II mRNA was decreased in high-grade (GSC 8–10) compared with low- grade (GSC 5– 6) cancer. IGF-II protein levels determined by IHC do not reflect mRNA expression levels in PCa.

Study	Year	Experiments	Samples	Statistical analysis	Results
IGF-IR					
Tennant	1996	**IHC** and **ISH** to compare the expression of IGF-IR and IGF-II in benign epithelium, HG-PIN and prostate adenocarcinoma	32 prostatectomy specimens	ANOVA, Fisher's exact test and paired t-tests	IGF-IR mRNA and protein was decreased in PIN and in PCa compared to benign epithelium (mRNA: $p<0.0001$; protein: $p<0.0004$).
Cardillo	2003	**IHC**, **ISH** and **qPCR** of IGF-I, IGF-II and IGF-IR protein and mRNA expression in PCa, PIN and NAP	Prostatectomy specimens Control: PIN and NAP	Mean +/- SEM, Chi-square, Student's paired t-test, Mann-Whitney U-test, Spearman rank correlation and linear regression and ANOVA	IGF-IR mRNA expression increased from NAP to PIN to PCa in the epithelial ($p<0.0001$) and stromal ($p=0.001$). IGF-IR protein increased in PCa than PIN and normal epithelial cells ($p<0.0001$)
Ryan	2007	**IHC** of IGF-IR expression in normal prostate epithelium and prostate cancer specimens	30 primary prostate tumours 5 locally recurrent androgen-independent tumors; 5 distant androgen-independent lymph node metastases Control: benign prostates from men without PCa	Mann Whitney test and Fisher exact test	IGF-IR protein was expressed in both normal prostate epithelium and PCa. No associations between the Gleason grade and absence or presence of the IGF-IR ($p =0.17$ for normal epithelium in men with cancer; $p= 0.26$ for PCa). No associations observed between stromal staining and tumour stage (T1c vs. T2–T4, $P =1.0$), race (white vs. non-white, $P=0.71$), age ($P= 0.49$), or PSA level ($P= 0.56$).
Massoner	2011	**IHC** and **qPCR** of IGF axis (IGF-I, IGF-II, IGFBP 1-6 and insulin receptor) in microdisseced tissue specimens of local PCa	Set 1: 20 local PCa specimens assigned according to their Gleason score used for laser microdissection; Set 2: 10 local PCa specimens; Set 3: 22 samples for IHC together with 20 samples from set 1 Control: Benign tissues from the prostatectomy specimens	Spearman's p test for correlation, Mann-Whitney U-test and student's t-test	IGF-IR mRNA was decreased in PCa compared with benign epithelial cells. .
Turney	2011	IHC of IGF-IR expression in serial prostate cancer specimens	18 patients who had undergone serial channel TURP at least 3 months apart. Controls: prostate cancer sections that had previously stained heavily for IGF-IR	Not stated	No correlation between Gleason sum score and IGF-1R protein. 6/7 patients with falling IGF-1R staining scores were responding to androgen deprivation therapy (confirmed by PSA response) between operations. 7/8 patients who had progression to androgen-independence between procedures, IGF-1R levels increased or remained high. 7/11 patients developed radiologically confirmed metastases between procedures showed stable or increasing IGF-IR staining.

Study	Year	Experiments	Samples	Statistical analysis	Results
IGF-IR (Continued)					
Hetzl	2012	IHC of IGF-IR expression in both prostatic stromal and epithelial.	45 prostatic samples from PCa patients obtained by radical prostatectomy Control: 15 prostatic samples were obtained from necropsied patients without a diagnosis of prostatic or other urological diseases	ANOVA and post-hoc Tukey's test	Increased IGF-IR protein levels in PCa and HG-PIN compared to normal and BPH group ($p<0.01$).
Zu	2013	**IHC** of IGF-IR expression in PCa to investigate if IGF-IR is a potential effect modifier for the association between PTEN expression and lethal prostate cancer risk.	651 men with PCa	Logistic regression multivariate Cox models and Wald test	High IGF-IR protein alone was associated fatal prostate cancer or distant metastasis (HR:13.8; 95% CI, 1.7–112.8). A significant negative interaction between PTEN and IGF-IR was found ($P_{interaction}=0.03$).
IGFBP-2					
Tennant	1996	**IHC** and **ISH** was carried out to compare expression of IGFBP-2 and 3 in PCa and HG-PIN	28 prostatectomy specimens from prostate adenocarcinoma Benign (n=28); HG-PIN (n=8); PCa (n=11)	ANOVA, Fisher's exact test, linear regression and paired t-tests	IGFBP-2 protein and mRNA was increased in PIN ($p<0.0003$) and PCa ($p<0.0003$).
Thrasher	1996	**IHC** of IGFBP-2 and IGFBP-3 protein expression in prostate tissues	24 patients who underwent radical prostatectomy for localized prostate adenocarcinoma	ANOVA and Fisher's protected least squared difference post-hoc test : compare IGFBP-2 and 3 among different groups.	IGFBP-2 protein was increased in PIN ($p<0.001$), and PCa ($p<0.001$) compared to normal epithelium and increased in PCa compared to PIN ($p <0.05$). No correlation between the stage and grade of prostate cancer and IGFBP-2 immunostaining intensity ($p = 0.69$ and 0.48, respectively) No correlation between preoperative serum PSA determinations and IGFBP-2 immunoreactivity ($p= 0.99$)
Figueroa	1998	IGFBP 1-6 RNA expression in benign and neoplastic prostate tissue detected by **RNAse protection assay**	23 consecutive radical prostatectomy specimens High Gleason scores (7-10): n=6; Low or intermediate scores (2-6): n=17 Control: Benign prostate tissue (Note: samples obtained from the same prostatectomy specimens)	One-tailed Student's t-test	Tumours with high Gleason score expressed higher IGFBP-2 RNA levels (0.4555 (CI: 0.395-0.515) vs 0.3 (CI: 0.256-0.356) ($p<0.002$). High Gleason score tumours had a 117% and 95% higher IGFBP-2/IGFBP-3 RNA expression ratio compared with benign and low grade tumours, respectively.

Study	Year	Experiments	Samples	Statistical analysis	Results
IGFBP-2 (Continued)					
Mita	2000	**qPCR** analysis of mRNA of IGF system	24 prostatectomy specimens after neoadjuvant hormone therapy No controls included.	Mann-Whitney U-test	IGFBP-2 mRNA was associated with pathologic stage (p=0.002), LN Mets (p=0.001), histologic differentiation (p=0.002) and serum PSA level (p=0.002) after hormone therapy.
Ambriosini-Spaltro	2001	**IHC** of IGFBP-2 in normal epithelium, HG-PIN and Prostate adenocarcinoma	Prostatectomy specimens (n=60) Group 1: Patients with bladder outlet obstruction Group 2: Patients hormonally untreated before surgery Group 3: Patients who underwent complete androgen ablation 3 months before surgery Control: HGPIN and normal epithelium	ROC curves: % of positive neoplastic cells Wilcoxon signed rank test: % of +ve neoplastic cells Spearman rank test: Gleason scores, pathologic stages and IHC scores	IGFBP-2 is expressed in the cytoplasm of untreated PCa and to a lesser extent in HG-PIN. IGFBP-2 is expressed in PCa and HG-PIN after complete androgen ablation, but to a lesser extent than in the untreated neoplasms. IGFBP-2 expression in the untreated specimens is lower in HG-PIN than in invasive PCa. IGFBP-2 was positive in PCa cases but not in benign prostatic tissues. No correlation between IGFBP2 with Gleason Grade or Tumour stage.
Richardsen	2003	**IHC** of IGFBP-2 expression in high-grade PIN, and PCa	193 radical prostatectomy specimens from patients with localized prostate adenocarcinoma Controls: BPH (n=14)	Fisher's exact probability test	Significant overexpression of IGFBP-2 in all instances of PIN and in more than 90% of cancers regardless of the grade. . The majority of cases with invasive carcinoma showed an overexpression of IGFBP-2 in >90% of the cancer cells. Extent and pattern of IGFBP2 expression was not correlated with Gleason grade.
Massoner	2011	**IHC** and **qPCR** of IGF axis (IGF-I, IGF-II, IGFBP 1-6 and insulin receptor) in microdisseced tissue specimens of local PCa	Set 1: 20 local PCa specimens assigned according to their Gleason score used for laser microdissection Set 2: 10 local PCa specimens Set 3: 22 samples for IHC together with 20 samples from set 1 Benign tissues Note: samples obtained from the prostatectomy specimens)	Spearman's p test for correlation, Mann-Whitney U-test and student's t-test	IGFBP-2 mRNA expression levels unchanged in prostate cancer tissue areas compared with benign prostate tissue areas.

Study	Year	Experiments	Samples	Statistical analysis	Results
IGFBP-3					
Tennant	1996	**IHC** and **ISH** was carried out to compare expression of IGFBP-2 and 3 in PCa and HG-PIN	28 prostatectomy specimens from prostate adenocarcinoma Benign (n=28); HG-PIN (n=8); PCa (n=11)	ANOVA, Fisher's exact test, linear regression and paired t-tests	IGFBP-3 mRNA was unchanged in benign epithelium, PIN and PCa. IGFBP-3 protein was increased in PIN ($p<0.0001$) but as decreased in malignant cells ($p<0.0001$).
Thrasher	1996	**IHC** of IGFBP-2 and IGFBP-3 protein expression in prostate tissues	24 patients who underwent radical prostatectomy for localized prostate adenocarcinoma	ANOVA and Fisher's protected least squared difference post-hoc test : compare IGFBP-2 and 3 among different groups.	IGFBP-3 protein was decreased in PCa carcinoma compared to normal epithelium ($p <0.0001$). IGFBP-3 protein increased in PIN compared to normal epithelium ($p<0.001$). No correlation between the stage and grade of prostate cancer and IGFBP-3 protein ($p = 0.88$ and 0.52, respectively) No correlation between preoperative serum PSA determinations and IGFBP-3 immunoreactivity ($p=0.21$).
Figueroa	1998	IGFBP 1-6 RNA expression in benign and neoplastic prostate tissue detected by **RNAse protection assay**	23 consecutive radical prostatectomy specimens High Gleason scores (7-10): n=6; Low or intermediate scores (2-6): n=17 Control: Benign prostate tissue from the same prostatectomy specimens	One-tailed Student's t-test	IGFBP-3 RNA levels lower in high Gleason score tumours ($p=0.05$).
Hampel	1998	**IHC** of IGFBP-3 in prostate adenocarcinoma	Study 1: 20 neoplastic prostates; Control: 6 normal prostates obtained from patients undergoing cystoprostatectomy for bladder cancer Study 2: 24 radical prostatectomy specimens from patients with clinically localized prostate adenocarcinoma Control: 8 normal prostates from organ donors or from patients undergoing cysto-prostatectomy for bladder cancer.	Conventional non-parametric tests, including the Wilcoxon sign rank test and the Mann Whitney U test. Mixed model was fit to the repeated measures data	IGFBP-3 protein was decreased in PCa compared to normal epithelium ($p<0.0001$). IGFBP-3 protein was not associated with Gleason grade, recurrence or LN mets.

Study	Year	Experiments	Samples	Statistical analysis	Results
IGFBP-3 (Continued)					
Linos	2009	**IHC** of IGFBP-3 expression in prostatic adenocarcinoma samples	199 prostatectomy specimens. No control specimens from healthy controls.	No stated	Cytoplasmic IGFBP-3 over expression was observed in 119/199 (60%) of all tumors. Within the non-treated subgroup (n=144), cytoplasmic IGFBP-3 over expression correlated with high tumor Gleason grade (Gleason score of 7 or more) [65% high grade versus 48% low grade, p=0.042]. Trend towards advanced stage, with 66% advanced stage tumors over expressing IGFBP3 protein versus 51% organ confined tumors, p=0.10).
Seligson	2013	**IHC** of nuclear and cytoplasmic IGFBP-3 protein expression in PCa	226 prostatectomy specimens of prostate adenocarcinoma obtained from randomly selected, hormone naïve patients Median Age: 65 (range: 46-76). Control: Matched benign (morphologically normal or hypertrophic) or in situ neoplastic lesions (PIN) obtained from prostate cancer patients	Kruskal-Wallis and Mann Whitney U tests: differences between nominal clinicopathologic prognostic variables. Chi-squared test: association of dichotomized IGFBP-3 versus nominal variables. Kaplan-Meier plots and Cox proportional Hazards regression models: association with recurrence-free time.	Higher IGFBP-3 cytoplasmic and nuclear staining in PCa than benign tissues (p<0.0001). Expression of nuclear IGFBP-3 was associated with disease recurrence as both a continuous p=0.0039, HR: 1.03; 95% CI: 1.01-1.06) and a dichotomized (p=0.0033; 2.51; 95% CI: 1.36-4.63) variable. Median recurrence-free time was 60 months for cases with high nuclear IGFBP-3 (n=71), and the median was not reached for cases with low nuclear IGFBP-3 (n=123) (Logrank p=0.0074). In patients with primary low-grade cancer, the presence of nuclear IGFBP-3 was even more predictive of tumor recurrence than in all cases (Logrank p=0.0007). In the low-grade group, IGFBP-3 mean positivity association with recurrence (p=0.0007, HR: 1.03; 95% CI: 1.03-1.10), and of dichotomized data (p=0.001; 5.44; 95% CI: 1.99-14.87).

Note: PCa: Prostate cancer; HG-PIN: High grade prostatic intraepithelial neoplasia; IHC: immunohistochemistry; ISH: in-situ hybridisation; NAP: normal adjacent counterpart; SEM: standard error of mean; qPCR: quantitative reverse-transcription polymerase chain reaction; BPH: Benign prostatic hyperplasia; ChIP: Chromatin immunoprecipitation; ab: antibody; ELISA: enzyme-linked immunosorbent assay; PSA: prostate specific antigen; TURP: transurethral resection of the prostate; MALDI-TOF: matrix-assisted laser desorption/ionization-time of flight; PCR: polymerase chain reaction; DRE: digital rectal examination; OR: Odds ratio; CI: confidence interval; LN mets: lymph node metastasis

Supplementary Table 4: Studies investigating whether genetic or epigenetic changes in the IGF system is associated with prostate cancer

Study	Year	Experiments	Samples	Experimental procedures	Statistical analysis	Results
IGF-I						
Cheng	2006	Investigated whether genetic variation at the IGF-I locus is associated with prostate cancer risk.	Sequencing: Men with advanced PCa (n=95) Genotyping Case-control study: Men with PCa (n=2320) Controls: men without PCa (n=2290)	Sequenced IGF-I exons in germline DNA Genotyping of tagged SNPs	Unconditioned logistic regression: association between PCa and IGF-I haplotypes and genotypes and permutation tests	Haplotype analysis revealed nominally statistically significant associations with PCa risk in each of the four haplotype blocks: haplotype 1B (OR = 1.21, 95% CI = 1.04 to 1.40), haplotype 2C (OR = 1.24, 95% CI = 1.06 to 1.44), haplotype 3C (OR = 1.25, 95% CI = 1.03 to 1.50), and haplotype 4D (OR = 1.19, 95% CI = 1.02 to 1.39). SNP3 (rs7978742) and SNP4 (rs7965399) was associated with prostate cancer risk (P_{trend} = .002). The CT genotype for SNP4 was associated with increased risk of prostate cancer, compared with the common homozygous TT genotype (OR = 1.25, 95% CI = 1.09 to 1.43; P = .001). This association was also statistically significant for non-advanced disease (OR = 1.32, 95% CI = 1.13 to 1.55; P<0.001).
Tsuchiya	2006	To examine the association of 13 genetic polymorphisms with survival of metastatic PCa patients.	111 PCa patients with bone metastasis at diagnosis and not received treatment	Polymerase chain reaction-restriction fragment length polymorphism or automated sequencer with genotyping	Kaplan-Meier curve, log rank test and cox proportional hazards model	Long allele (over 18 [CA] repeats) of insulin-like growth factor-I (IGF-I) was significantly associated with a worse cancer-specific survival (P =0.025). Long allele of IGF-I polymorphisms was an independent risk factor for death (HR: 2.01: 95% CI: 1.12-3.62;p =0.019).
Tsuchiya	2013	To evaluate the association of polymorphisms in 3 linkage disequilibrium blocks of IGF-I on survival of metastatic PCa patients.	215 patients with bone metastasis at initial presentation	Polymerase chain reaction-restriction fragment length polymorphism or automated sequencer with genotyping	Kaplan-Meier curve, log rank test and cox proportional hazards model	CA repeat polymorphism, rs12423791 and rs6220 are associated with cancer-specific survival (p=0.013, 0.014 and 0.014, respectively). Haplotype in LD block 3 was significantly associated with cancer–specific survival (p=0.0003). Patients with all the risk factors (19-repeat allele, C allele of rs12423791, or C-T haplotype) had a significantly shorter cancer specific survival than those with 0-2 of the risk factors (p=0.0003).
Johansson	2007	To investigate what extent genetic variation in the IGF-I gene is related to prostate cancer risk	Men with PCa (n=2863) Controls: men randomly selected from the Swedish population (n=1737)	Genotyping by 5' nuclease assay	Likelihood-ratio test and permutation testing	Common haplotypes in the block covering the 3 region of the IGF1 gene showed significant global association with prostate cancer risk (p = 0.004), with TCC haplotype giving an odds ratio of 1.46 (95% CI 5 1.15–1.84, p = 0.002).

Study	Year	Experiments	Samples	Experimental procedures	Statistical analysis	Results
IGF-I (Continued)						
Chang	2013	To investigate the association of 4 common SNPs in IGF-I and IGF-IR with age, PSA, Gleason score, surgical margin, lymph node metastasis and PSA recurrence	320 localised prostate cancer patients receiving radical prostatectomy	Genomic DNA was extracted from peripheral blood of patients. Genotyping (Sequenom iPLEX MALDI-TOF mass spectrometry	Logistic regression analyses and Cox proportional hazards regression: association of individual SNP alleles, genotypes and haplotypes with clinic pathological characteristics Multi-factor dimensionality reduction (MDR) analysis: interaction between SNPs and PSA recurrence.	IGF-I rs2946834 alleles/genotypes and an IGF-I specific haplotype AT, containing the minor allele of rs2946834, were associated with higher risk of having advanced-stage prostate cancer (OR: 1.58; 95 % CI: 1.05–2.38; p= 0.027). IGF-I haplotype AT was associated with an increased risk of having positive surgical margin after RP (OR, 1.65; 95 % CI:1.06–2.58; p= 0.03).
IGF-II						
Lai	2005	Investigate whether polymorphism of IGF-II could be used as a genetic marker for risk of PCa.	Patients with PCa (n=96); Controls: Healthy male volunteers from the same geographic area (n=121).	PCR using primers for IGF-II gene exon 9	Chi-square test	No significant difference between distribution of IGF-II gene C/T polymorphism between the healthy control group and the patients with PCa (p=0.78). No significant difference in the distribution of the IGF-II gene C/T polymorphism between individuals younger than 70yrs and those older than 71yrs (p=0.5).
Hu	2006	To determine whether the M6P/IGF-IIR gene is inactivated in PCa.	43 patients with PCa treated with radical prostatectomy	Regions of tumour, normal prostate and PIN were identified and cells were excised by laser capture microdissection. DNA segments amplified with PCR	Pearson chi-squared test and ANOVA: difference between groups Kaplan-Meier curve: disease-free survival	M6P/IGF-IIR gene was polymorphic in 83.7% (36/43) of patients. 41.7% (15/36) of these informative patients had loss of heterozygosity (LOH) in the tumor tissue. 11/15 patients with LOH in malignant tissue also had HG-PIN. Of these 63.6% (7/11) also had LOH in HG-PIN tissue. There was no significant difference in age, PSA levels, stage, Gleason score, proliferative index, cancer involvement, lymphatic/vascular invasion and perineural invasion between the groups with or without LOH. No significant difference in disease-free survival between the groups of with or without LOH.

Study	Year	Experiments	Samples	Experimental procedures	Statistical analysis	Results
IGF-II (Continued)						
Lui	2006	To explore the genomic imprinting of IGF-II in PCa and its correlation to disease progression.	PCa (n=41), BPH (n=27) and normal prostate tissue (n=13)	PCR-RFLP	Fisher's exact test; t-test; Kaplan-Meier and log rank test	Rates of heterozygote of IGF-II DNA were 70.7% in PCa, 55.5% in BPH and 61.5% in normal prostate tissue group. Occurrence rate of LOI of IGF-II was higher in PCa than in BPH and normal tissue (p=0.05). LOI of IGF-II had no correlation to age, PSA, presence of bone metastasis, and cell differentiation before endocrinotherapy. After androgen blockade, the 1 year progression free survival rate was lower in patient s with LOI of IGF-II tan in patients without LOI of IGF-I (p=0.04).
Fu	2008	Determined whether normal imprint is altered for the *IGF-2* gene with aging in the prostate.	Prostate tissues from C57/B6 mice (containing a Cast I*GF-2-H19* allele) Histologically normal human prostate specimens from men without cancer (n=40) or men with cancer (n=25).	qPCR, DNA methylation sequencing, ChIP	Not stated	Significant loss of imprinting (LOI) for *IGF-2* in the dorsolateral prostate (DLP) beginning at 11 months compared to young sexually mature mice (3 months). LOI is associated with increase in *IGF-2* mRNA and protein expression. siRNA mediated down-regulation of CTCF induced LOI in prostate cells. No alteration in LOI of IGF-2 in the ventral prostate or non prostate tissues. LOI in histologically normal prostate tissues from men with cancer is significantly greater than men without cancer (p=0. 02).
Paradowska	2009	Analysed DNA methylation and histone modifications in the differentially methylated region (DMR) of IGF-II/H19 in benign prostate hyperplasia (BPH) and prostate carcinoma (PCa).	30 prostate radical prostatectomy or cystoprostatectomy Control: 17 BPH surrounding tumors	Sodium bisulfite treatment and DNA sequencing of genomic DNA The methylation pattern of 17 CpGs within 227 bp of the H19 fragment was characterized from each DNA sample. ChIP	Mann-Whitney test	All BPH samples demonstrated >80% methylation of CpGs while 41% of CpGs were methylated in 9 out of 30 PCa specimens. Statistically significant differences in the methylation state was found between PCa and BPH groups, especially in the differentially methylated region (DMR) of H19 (p<0.0001) and in the imprinting control region (ICR) (p=0.0034), which corresponds to CTCF binding domain. ChIP assay revealed that dimethyl H3K9 is associated with the ICR of IGF-II/H19 in BPH, but not in PCa (p<0.0001).

Study	Year	Experiments	Samples	Experimental procedures	Statistical analysis	Results
IGF-II (Continued)						
Bhusari	2011	To define whether IGF-2 LOI in histologically normal prostate tissues in relationship to tumour foci and gene expression	Prostatectomy samples containing tumour and associated normal tissue (n=18) Controls: Normal prostate samples without any associated tumours from age-matched men.	Fluorescent primer extension (FluPE), qPCR, DNA methylation analysis	Spearman's correlation; two-tailed t-test	Marked IGF-2 LOI in adjacent tumour associated tissues (39 ±3.1%) but not in tissues distant (38 ±5.3%) from tumour foci (45±2.9%). IGF-2 imprinting correlated with IGF-2 expression in adjacent tumour associated tissues but not within the tumour foci. Hypomethylation of IGF-2 DMRO region correlated with decreased IGF-2 expression in tumours ($p<0.01$). The expression of IGF-2 and H19 gene were increased in adjacent and distant tissues compared to tumours ($p<0.05$).
Belharazem	2012	To investigate levels of IGF-II protein levels and IGF-II 820G/A genotype whether loss of imprinting (LOI) of IGF-II in normal circulating peripheral blood lymphocytes can predict increased PCa risk	113 blood samples of patients with a history of radical prostatectomy for PCa Controls: volunteer blood donors	ELISA: serum IGF-II and IGFBP-3 levels Restriction-fragment length polymorphism: heterozygosity at ApaI-sensitive 820G>A locus on exon 7 of IGF-II gene LOI or retention of imprinting (ROI): cDNA amplification from heterozygous cases by a nested RT-PCR method. Bisulfite-DNA sequencing: Methylation status of IGF-II imprinting control region (ICR)	Chi-square test, Mann–Whitney U test and Spearman's test	Among men with a history of PCa, the 820G/A genotype was significantly more frequent than among healthy control persons (50.5% in PCa patients vs 43% in controls; OR 1.92; 95% CI: 1.22–3.02, p=0.005). LOI in PCa patients was significantly more frequent (16/41 cases (39%), P=0.03). Higher degree of methylation in samples with LOI than in ROI in both PCa patients and controls. All ICRs in samples of RPE patients, irrespective of the imprinting status, showed a higher degree of methylation compared with control samples.

Study	Year	Experiments	Samples	Experimental procedures	Statistical analysis	Results
IGFBP-2						
Hu	2006	To determine whether the M6P/IGF-IIR gene is inactivated in PCa.	43 patients with PCa treated with radical prostatectomy	Regions of tumour, normal prostate and PIN were identified and cells were excised by laser capture microdissection. DNA segments amplified with PCR	Pearson chi-squared test and ANOVA: difference between groups Kaplan-Meier curve: disease-free survival	M6P/IGF-IIR gene was polymorphic in 83.7% (36/43) of patients. 41.7% (15/36) of these informative patients had loss of heterozygosity (LOH) in the tumor tissue. 11/15 patients with LOH in malignant tissue also had HG-PIN. Of these 63.6% (7/11) also had LOH in HG-PIN tissue. No significant difference in age, PSA levels, stage, Gleason score, proliferative index, lymphatic/vascular invasion, and disease-free survival between the groups with or without LOH.
Paradowska	2009	Analysed DNA methylation and histone modifications in the differentially methylated region (DMR) of IGF-II/H19 in benign prostate hyperplasia (BPH) and prostate carcinoma (PCa).	30 prostate radical prostatectomy or cystoprostatectomy Control: 17 BPH surrounding tumors	Sodium bisulfite treatment and DNA sequencing of genomic DNA The methylation pattern of 17 CpGs within 227 bp of the H19 fragment was characterized from each DNA sample. ChIP	Mann-Whitney test	All BPH samples demonstrated >80% methylation of CpGs while 41% of CpGs were methylated in 9 out of 30 PCa specimens. Statistically significant differences in the methylation state was found between PCa and BPH groups, especially in the differentially methylated region (DMR) of H19 ($p<0.0001$) and in the imprinting control region (ICR) ($p=0.0034$), which corresponds to CTCF binding domain. ChIP assay revealed that dimethyl H3K9 is associated with the ICR of IGF-II/H19 in BPH, but not in PCa ($p<0.0001$).
IGFBP-3						
Okugi	2006	Investigated whether the methylation status of IGFBP-3 promoter in prostate tissues influences the progression and prognosis of prostate cancer.	PCa patients (n=38) Controls: BPH (n=57)	Bisulfite modification and methylation-specific PCR of genomic DNA Hep-methylation status and NSCLC-methylation status	Chi-squared test: distribution of methylation frequency of IGFBP-3 promoter region Unconditional logistic regression: OR and 95% CI	No significant difference in the methylation frequency of the IGFBP-3 promoter between cases and controls (OR:1.53; 95% CI: 0.85-5.56; $p=0.15$) for Hep-primer method; OR=3.24; 95% CI: 0.46-15.42; $p=0.21$ for NSCLC-primer method). No statistically significant association between the hypermethylation of IGFBP-3 promoter and clinical stage or Gleason score.

Study	Year	Experiments	Samples	Experimental procedures	Statistical analysis	Results
IGFBP-3 (Continued)						
Perry	2007	Investigated the methylation pattern of IGFBP-3 in benign, pre-invasive and cancerous prostate tissues	40 prostatectomy specimens; 39 primary tumours (USA); 14 HG-PIN lesions (from 79 patients) with PCa. Control: histologically normal adjacent tissue from PCa patients and BPH lesions	Bisulfite modification of genomic DNA and quantitative methylation specific PCR	Fisher's exact test Kruskal-Wallis one way ANOVA test and Wilcoxon-matched pairs test	IGFBP3 promoter was completely unmethylated in the histologically normal prostate samples. In the BPH samples, the frequencies of IGFBP-3 promoter methylation were significantly less than detected in tumours (P<0.0001). IGFBP-3 promoter methylation was only detected in HGPIN samples from patients whose adjacent tumour was also methylated The frequency of IGFBP3 methylation in HGPIN was not statistically different from tumour (p=0.383). Methylation of IGFBP-3 promoter was detected in significantly more in tumours with Gleason score ≥7, than ≤6 (p=0.01), but was not significantly correlated with TNM classification or PSA level.
Johansson	2009	Analysed genetic variation within genes coding for IGFBP in relation to prostate cancer incidence and survival.	Genotyping analysis: PCa cases (n=2774); Controls: men randomly selected from the Swedish population (n=1736)	Genotyping by 5' nuclease assay ELISA: plasma total and intact IGFBP-3 levels	Conditional logistic regression: odds ratio Cox proportional hazards: survival analysis Likelihood ratio test	No association between the IGFBP-3 genetic variants and prostate cancer incidence or survival. The rare allele of the IGFBP-3 SNP rs2854744 was associated with elevated plasma levels of total IGFBP-3 (P_{trend} =9x 10^{-8}), but not intact IGFBP3 ($P_{trend=}$0.16).

Note: PCa: Prostate cancer; HG-PIN: High grade prostatic intraepithelial neoplasia; IHC: immunohistochemistry; NAP: normal adjacent counterpart; SEM: standard error of mean; qPCR: quantitative reverse-transcription polymerase chain reaction; BPH: Benign prostatic hyperplasia; ChIP: Chromatin immunoprecipitation; ab: antibody; ELISA: enzyme-linked immunosorbent assay; PSA: prostate specific antigen; TURP: transurethral resection of the prostate; MALDI-TOF: matrix-assisted laser desorption/ionization-time of flight; PCR: polymerase chain reaction; DRE: digital rectal examination; OR: Odds ratio; CI: confidence interval ; PCR-RFLP: Polymerase chain reaction-based restrictive fragment length polymorphism

Supplementary Table 5: Studies investigating circulating levels of IGF system and prostate cancer included as supporting evidence.

Study	Year	Experiments	Samples	Experimental procedures	Statistical analysis	Results
IGF-I						
Tricoli	1999	To determine the overall plasma levels of IGF-I in men at higher risk of PCa development and to investigate the relationships between demographic and IGF-I levels	105 men (63 African American (AA) and 42 White) with no personal history of PCa but have at least one 1st degree relative diagnosed with PCa.	ELISA	Wilcoxon test; Spearman correlation coefficient; linear regression;	Mean plasma level of IGF-I was not significantly different between AA (162.3ng/ml) and white (172.1ng/ml) men (p=0.42). Inverse relationship between IGF-I plasma levels and age (p=0.008).
Baffa	2000	Relation between serum IGF-I and PCa	57 patients who underwent radical prostatectomy for adenocarcinoma. Serum samples collected before radical prostatectomy (T0) or 6 months after radical prostatectomy (T6) Controls: 39 age-matched controls	Active IGF-I Elisa Kit (Diagnostic systems Lab)	Welch's t-test and paired t-test	Serum IGF-I levels were lower in patients with PCa (124.6± 58.2ng/ml) compared to the control (157.5± 70.8ng/ml) (p=0.0192). Mean serum IGF-I levels for case patients at T0 (124.91± 58.6ng/ml) was lower than patients in the T6 group (148.49± 57.2ng/ml) (p=0.0056).
Shariat	2000	Investigate pre-operative levels of IGF-I plasma levels in patients with clinically localize PCa	120 patients who underwent radical prostatectomy for clinically localized prostatic adenocarcinoma. Control: Healthy patients without PCa (n=20). (No prior history of any cancer or chronic disease, a normal digital rectal examination, and a PSA<2ng/ml).	DSL-105600 Active-IGF-I ELISA assay	ANOVA: means among patient groups. Spearman's rank correlation coefficient: compare ordinal and continuous variables Logistic regression multivariate analysis of binary outcomes.	From univariate analysis, pretreatment IGF-I levels did not correlate with age (p=0.89), preoperative PSA (p=0.28), pathologic Gleason score (p=0.49) and pathologic stage (p=0.56). In both a univariate and a multivariate logistic regression analysis that included preoperative IGF-I, preoperative PSA, clinical stage, and biopsy Gleason score, IGF-I levels did not predict organ- confined disease (P =0.56, P = 0.4165, respectively).
Shariat (Cont.)					Kaplan-Meier Curves: Survival analysis Cox Proportional hazards: time to recurrence	No significant difference for PSA progression-free survival between patients with high IGF-I levels (≥151.1ng/ml) and patients with low IGF-I levels (<151.1ng/ml) (P= 0.76). IGF-I levels in radical prostatectomy patients were not significantly higher than those in healthy subjects or in patients with metastatic disease (p=084).
Stattin	2001	To investigate if increased plasma leptin levels are associated with development of PCa.	PCa Patients (n=149); Controls: Matched men without cancer (n=298)	Immunoradiometric assays	Pearson correlation analysis; Univariate and multivariate logistic regression analysis	Adjustments for IGF-I or IGFBP 1-3, (either as continuous or categorical variables) in separate and combined multivariate models did not attenuate the increased risk associated with moderately elevated leptin levels.

Study	Year	Experiments	Samples	Experimental procedures	Statistical analysis	Results
IGF-I (Continued)						
Yu	2001	To determine changes in IGF-I, IGFBP-2 and IGFBP-3 levels in serial post-operative serum samples from PCa patients with and without relapse	PCa Patients (n=148) Patients who developed recurrence (n=38) Controls: patients who remained in remission (n=40)	ELISA to measure IGF-I, IGFBP-2 and IGFBP-3 in serum samples	Wilcoxon rank-sum test; Friedman test; Page's L test; generalized linear model (GLM)	No difference in IGF-I levels between cases and controls (p=0.28).
Latif	2002	To assess the relationships between IGF-I and PCa disease stage	Patients with BPH (n=17), stage T1/T2 PCa (n=15), T3/T4 cancer (n=16) and metastatic PCa (n=12)	Immuno-enzymometric assay: IGF-I ELISA: IGFBP-3	Anova (Kruskal-Wallis) and Mann-Whitney U-test	IGF-I concentrations were similar between patients with BPH and those with cancer. No correlation between age and IGF-I concentration.
Oliver	2003	Assess whether serum levels of IGFs and IGFBPs were associated with grade, serum PSA and clinical stage.	224 men (50-70yrs) with screen-detected prostate cancer identified via population-based case-finding in three UK centres All had total PSA ≥3ng/ml No healthy controls	IGF-I & -II (ELISA, DSL) IGFBP-2 (RIA, DSL), IGFBP-3 (RIA, 'in-house' assay) the molar ratio of IGF-I:IGFBP-3 (a measure of IGF-I bioavailability) was derived.	Not stated	Geometric mean levels of IGF-I did not vary by stage or grade but were higher in cases with a higher PSA (PSA 3-5ng/ml geometric mean (CI) IGF-I 126.5ng/ml (121.5-133.0), PSA 20+ng/m1 IGF-I 144.0 (129.0- 159.2), P_{trend}=0.05). The IGF-I:IGFBP-3 molar ratio did not differ by clinical stage, but was significantly higher in cases with higher PSA (PSA 3-5ng/ml geometric mean (CI) molar ratio 19.8% (18.6-21.0), PSA 20+ng/ml molar ratio 23.2% (20.6-26.4), P_{trend}=0.03) The IGF-I:IGFBP-3 molar ratio was higher in men with higher grade tumours, (Gleason <7 geometric mean (CI) molar ratio 19.8% (18.8-21.0), Gleason 2:7 geometric mean (CI) molar ratio 22.3% (20.4-24.2), P=0.03)
Woodson	2003	To evaluate the association between pre-diagnostic levels of IGF-I and IGFBP-3 and PCa risk in a nested case-control study (RAS) To examine changes in serum IGF-I and IGFBP-3 levels over time (SSS)	RAS: PCa (n=100); Controls: randomly selected members from cohort SSS: PCa (n=21); Controls: trial participants who had no cancer diagnosed (except non-melanoma skin cancer) over the full period of study follow-up and had two serum draws at least 1 year apart.	ELISA	Logistic regression and paired t-test	No significant association between prostate cancer risk and either serum IGF-I (OR: 0.52; 95% CI, 0.23–1.16) after adjusting for age, BMI, intervention group assignment. Ratio of IGF-I: IGFBP-3 had borderline significant inverse association with PCa risk (p=0.06). The cases had an average 18% increase in serum IGF-I levels compared with a 4% decrease among controls (P=0.02).

Study	Year	Experiments	Samples	Experimental procedures	Statistical analysis	Results
IGF-I (Continued)						
Tu	2004	To investigate the levels of IGF-1 and IGFBP-3 in bone marrow aspirates and plasma samples of men with advanced prostate cancer	42 patients with widespread bone metastases (n-22) and without bone metastasis (n=20)	ELISA	Not stated	Levels of IGF-I and IGFBP-3 were lower in bone marrow supernatant than in plasma. Bone marrow supernatant: median IGFBP-3 levels were significantly lower in the Met group (834.5ng/ml) than in the Non-Met group (1650ng/ml) (p=0.0001). No correlation between IGF-I levels and metastasis.
Nam	2005	To determine whether high serum IGF-I levels are associated with precancerous lesions of the prostate To compare serum IGF-I and IGFBP-3 levels between men with PCa and those without cancer or with HG-PIN	Cases: Patients with HGPIN from prostate biopsy. Controls: no evidence of adenocarcinoma of the prostate or HG-PIN (2 or more negative biopsies)	ELISA	Not stated	The mean serum IGF-I level for patients with HGPIN (130.2 ng/mL) was significantly higher than for controls (118.8 ng/mL, P = 0.01). The crude odds ratio for having HGPIN for patients with the highest quartile of serum IGF-I level compared with the lowest quartile group was 1.95 [95% confidence interval (CI), 1.0-3.7; P = 0.04) The mean IGF-I level for patients with cancer was 119.4 ng/mL (n = 483) and was not significantly different from the 205 patients in the control group (118.8 ng/mL, P = 0.85).
Woongeol	2007	Case-control study to investigate the association between serum IGF-I and IGFBP-3 levels and prostate cancer risk	330 men (165 cases treated by radical prostatectomy and 165 healthy age-matched controls).	Not stated	Conditional logistic regression	Risk of PCa not related to IGF-I and IGF-1:IGFBP-3 molar ratio.
Ito	2009	IGF-1 and IGFBP-3 kinetics	78 cases with baseline PSA<4ng/ml and diagnosed with PCa after undergoing at 3 times of screening Control: 156 age-adjusted and baseline PSA-adjusted men without prostate cancer and screened at least 3 times. Men with PSA velocity <0.2ng/ml/yr was recommended for selection.	Serum IGF-1 and IGFBP-3 were measured using serum samples at initial, intermediate and last screening visits in each participant.	Not stated	There was no significant difference in the baseline IGF- 1, baseline IGFBP-3, IGF-1 velocity and IGFBP-3 velocity between the case and the control group.

Study	Year	Experiments	Samples	Experimental procedures	Statistical analysis	Results
IGF-I (Continued)						
Mucci	2010	To investigate the levels of IGF-I and IGFBP-3 in plasma samples from PCa patients	545 incident cases; Controls: 545 matched-controls	ELISA	Conditional logistic regression models	No association between free IGF-I and prostate cancer risk (RR, 0.9; 95% CI: 0.6-1.3)
Rowlands	2012	Investigated associations of circulating IGF-I, IGF-II, IGFBP-2 and IGFBP-3 with all-cause and PCa mortality in men with clinically identified PCa, stratified by whether localised (stage T1 or T2) or advanced (T3, T4, N1 or M1) at diagnosis.	396 men with PCa	In-house radioimmunoassay (RIA): For IGF-I, IGF-II and IGFBP-3 ELISA: total IGF-I, IGF-II or IGFBP-3	Age-adjusted linear regression models, likelihood ratio test and Cox regression hazards regression	In men with advanced cancer, IGF-I was positively associated (HR 1.20; 95% CI: 0.96, 1.49; p = 0.11) and IGFBP-3 was inversely associated (HR 0.84; 95% CI: 0.70, 1.01; p = 0.07) with all-cause mortality after controlling for age, treatment status, smoking, prostate-specific antigen and Gleason grade at diagnosis. IGF-I was positively associated with prostate cancer mortality in advanced cases (HR 1.23; 95% CI: 0.94, 1.62; p = 0.13). In advanced cancers, associations of IGF-I with all-cause (HR 1.68; 95% CI: 1.28, 2.23; p<0.001) and prostate cancer–specific (HR 1.59; 95% CI: 1.11, 2.28; p = 0.01) mortality strengthened (and were conventionally statistically significant) and controlling for IGFBP-3.
IGF-II						
Oliver	2003	Assess whether serum levels of IGFs and IGFBPs were associated with grade, serum PSA and clinical stage.	224 men (50-70yrs) with screen-detected prostate cancer identified via population-based case-finding in three UK centres. All had total PSA ≥3ng/ml No healthy controls	IGF-I & -II (ELISA, DSL); IGFBP-2 (RIA, DSL); IGFBP-3 (RIA, 'in-house' assay) Molar ratio of IGF-I:IGFBP-3 (a measure of IGF-I bioavailability) was derived.	Not stated	After adjustment for age and centre, geometric mean levels of IGF-II did not differ by disease stage, grade or PSA.
Belharazem	2012	To investigate levels of IGF-II protein levels and IGF-II 820G/A genotype whether loss of imprinting (LOI) of IGF-II in normal circulating peripheral blood lymphocytes can predict increased PCa risk	113 blood samples of patients with a history of radical prostatectomy for PCa Controls: volunteer blood donors	ELISA: serum IGF-II and IGFBP-3 levels	Chi-square test, Mann–Whitney U test and Spearman's test	In contrast to controls, IGF-II levels in all PCa patients were increased and appeared uncoupled from the imprinting status (p=0.9). IGF-II protein levels both in patients and in controls were tightly correlated with IGFBP-3 levels (r=0.8; p<0.0001).

Study	Year	Experiments	Samples	Experimental procedures	Statistical analysis	Results
IGFBP-2						
Yu	2001	To determine changes in IGF-I, IGFBP-2 and IGFBP-3 levels in serial post-operative serum samples from PCa patients with and without relapse	PCa Patients (n=148) Patients who developed recurrence (n=38) Controls: patients who remained in remission (n=40)	ELISA to measure IGF-I, IGFBP-2 and IGFBP-3 in serum samples	Wilcoxon rank-sum test; Friedman test; Page's L test; generalized linear model (GLM)	Lower serum levels of IGFBP-2 in cases than in controls ($p<0.05$). In sequential samples, IGFBP-2 levels increased over time in controls ($p=0.014$) but not in cases ($p=0.53$).
Oliver	2003	Assess whether serum levels of IGFs and IGFBPs were associated with grade, serum PSA and clinical stage.	224 men (50-70yrs) with screen-detected prostate cancer identified via population-based case-finding in three UK centres. All had total PSA ≥3ng/ml No healthy controls.	IGF-I & -II (ELISA, DSL); IGFBP-2 (RIA, DSL); IGFBP-3 (RIA, 'in-house' assay) Molar ratio of IGF-I:IGFBP-3 (a measure of IGF-I bioavailability) was derived.	Not stated	After adjustment for age and centre, geometric mean levels of IGFBP-2 did not differ by disease stage, grade or PSA.
IGFBP-3						
Smith	1999	Compared concentrations of IGFBP-3 and PSA in bone metastases and measured serum IGFBP-3 in patients with changing PSA concentrations.	Metastatic bone tissues from patients with PCa (n=6) and patients with breast cancer (n=5)	Western blot: IGFBP-3 concentrations in metastatic tissue ELISA: serum IGFBP-3 levels	Mann-Whitney test	IGFBP-3 tissue concentrations in PSA-positive bone metastases from patients with PCa were lower compared to PSA-negative bone metastases from patients with breast cancer ($p=0.0081$). Inverse correlation between serum PSA and IGFBP-3 concentrations in patients with PCa during period of therapeutic response or disease progression.
Tricoli	1999	To determine the overall plasma levels of IGF-I in men at higher risk of PCa development and to investigate the relationships between demographic and IGF-I levels	105 men (63 African American (AA) and 42 White) with no personal history of PCa but have at least one 1st degree relative diagnosed with PCa.	ELISA	Wilcoxon test; Spearman correlation coefficient; linear regression;	Mean plasma level of IGFBP-3 was lower in AA (2789 ng/ml) than in white (3216ng/ml) men ($p=0.005$). No correlation between IGFBP-3 plasma levels and age.
Yu	2001	To determine changes in IGF-I, IGFBP-2 and IGFBP-3 levels in serial post-operative serum samples from PCa patients with and without relapse	PCa Patients (n=148) Patients who developed recurrence (n=38) Controls: patients who remained in remission (n=40)	ELISA to measure IGF-I, IGFBP-2 and IGFBP-3 in serum samples	Wilcoxon rank-sum test; Friedman test; Page's L test; generalized linear model (GLM)	Lower serum levels of IGFBP-3 in cases than in controls ($p<0.05$).

Study	Year	Experiments	Samples	Experimental procedures	Statistical analysis	Results
IGFBP-3 (Continued)						
Latif	2002	To assess the relationships between IGF-I and PCa disease stage	Patients with BPH (n=17), stage T1/T2 PCa (n=15), T3/T4 cancer (n=16) and metastatic PCa (n=12)	Immuno-enzymometric assay: IGF-I ELISA: IGFBP-3	Anova (Kruskal-Wallis) and Mann-Whitney U-test	IGFBP-3 concentrations were similar between patients with BPH and those with cancer. Age was correlated with IGFBP-3 concentrations (r=-0.4; p=0.008).
Oliver	2003	Assess whether serum levels of IGFs and IGFBPs were associated with grade, serum PSA and clinical stage.	224 men (50-70yrs) with screen-detected prostate cancer identified via population-based case-finding in three UK centres. All had total PSA ≥3ng/ml No healthy controls	IGF-I & -II (ELISA, DSL); IGFBP-2 (RIA, DSL); IGFBP-3 (RIA, 'in-house' assay); Molar ratio of IGF-I:IGFBP-3 (a measure of IGF-I bioavailability) was derived.	Not stated	After adjustment for age and centre, geometric mean levels of IGFBP-3 did not differ by disease stage, grade or PSA.
Tu	2004	To investigate the levels of IGF-1 and IGFBP-3 in bone marrow aspirates and plasma samples of men with advanced prostate cancer.	42 patients with widespread bone metastases (n-22) and without bone metastasis (n=20)	ELISA	Not stated	IGFBP-3 levels in the bone marrow supernatant inversely correlated with serum alkaline phosphatase (p=0.0003) and PSA (p=0.02).
Nam	2005	To determine whether high serum IGF-I levels are associated with precancerous lesions of the prostate To compare serum IGF-I and IGFBP-3 levels between men with PCa and those without cancer or with HG-PIN	Cases: Patients with HGPIN from prostate biopsy. Controls: no evidence of adenocarcinoma of the prostate or HG-PIN (2 or more negative biopsies)	ELISA	Not stated	The mean IGFBP-3 level was slightly higher for patients with HGPIN (2,393.9 ng/mL) compared with controls (2,276.0, P = 0.06). The crude odds ratio for having HGPIN for patients with the highest quartile of serum IGFBP-3 level compared with the lowest quartile group was 2.04 (95% CI, 1.1- 3.9; P = 0.03). The mean IGFBP-3 level for patients with cancer (2,222.7 ng/mL) was also not significantly different to the control group (2,276.0 ng/mL, P = 0.26).
Woongeol	2007	Case-control study to investigate the association between serum IGF-I and IGFBP-3 levels and prostate cancer risk	330 men (165 cases treated by radical prostatectomy and 165 healthy age-matched controls).	Not stated	Conditional logistic regression	Strong inverse association between IGFBP-3 and PCa risk. Men in highest quartile of IGFBP-3 had 88% reduced risk of PCa compared with men in the lowest quartile (OR=0.12; 95% CI: 0.05-0.64; p<0.01). 48% and 76% reduced risk of aggressive prostate cancer in 3rd and 4th quartile of IGFBP-3 levels compared to 1st quartile.

Note: PCa: Prostate cancer; HG-PIN: High grade prostatic intraepithelial neoplasia; IHC: immunohistochemistry; NAP: normal adjacent counterpart; SEM: standard error of mean; qPCR: quantitative reverse-transcription polymerase chain reaction; BPH: Benign prostatic hyperplasia; ChIP: Chromatin immunoprecipitation; ab: antibody; ELISA: enzyme-linked immunosorbent assay; PSA: prostate specific antigen; TURP: transurethral resection of the prostate; MALDI-TOF: matrix-assisted laser desorption/ionization-time of flight; PCR: polymerase chain reaction; DRE: digital rectal examination; OR: Odds ratio; CI: confidence interval

Supplementary Table 6 - GRADE assessment of studies of milk and IGF-I levels

Quality assessment	**Rating**	**Adjustment to rating**	**Notes**
No of studies/starting rating	4 human RCTs, 1 intervention study and 24 observational studies	2	Larger number of observational studies with a small number of RCTs, therefore start with score of 2
Factors decreasing confidence			
Limitations in study design (risk of bias)	Not serious	0	Most studies are moderate or unclear RoB
Inconsistency	Not serious	0	Just one outlier, but can be explained due to outcome being measured 65 years after exposure
Indirectness	Not serious	0	All studies looked at the effect of milk/dairy products on IGF1 levels
Imprecision	Not serious	0	Many large studies
Publication bias	Serious	-1	Although not clearly evident there is likely to be publication bias in this area of research
Factors increasing confidence			
Strength of association	Substantial	+1	Combined p-value is very low.
Dose-response		0	Studies were not able to examine this robustly (RCTs based on a single dose/intervention, or food frequency questionnaires)
Confounders likely to minimise the effect		0	Unable to rule out confounding, may play a role
Final numerical rating of quality of evidence		2	
Statement of quality of evidence		There is currently a low level of evidence linking milk with IGF-I levels and this suggests a positive association.	

Supplementary Table 7 - GRADE assessment of studies of milk and IGF-II levels

Quality assessment	**Rating**	**Adjustment to rating**	**Notes**
No of studies/starting rating	3 observational studies	2	Only 3 studies all observational, although large population sizes
Factors decreasing confidence			
Limitations in study design (risk of bias)	Not serious	0	1 low, 2 unclear
Inconsistency	Not serious	0	Difficult to determine due to the small number of studies, but no strong evidence of inconsistency
Indirectness	Not serious	0	All studies looked at the effect of milk/dairy products on IGFII levels
Imprecision	Serious	-1	Based on just 3 studies, two of which had wide confidence intervals
Publication bias	Serious	-1	Although not clearly evident there is likely to be publication bias in this area of research
Factors increasing confidence			
Strength of association		0	Strong association but this was in just one study
Dose-response		0	Not robust
Confounders likely to minimise the effect		0	Unable to rule out confounding, may play a role
Final numerical rating of quality of evidence		1	
Statement of quality of evidence		There is currently a very low level of evidence linking milk with IGF-II levels and this suggests a positive association.	

Supplementary Table 8 - GRADE assessment of studies of milk and IGFBP-I levels

Quality assessment	**Rating**	**Adjustment to rating**	**Notes**
No of studies/starting rating	2 observational studies	2	Only 2 studies both observational
Factors decreasing confidence			
Limitations in study design (risk of bias)	Not serious	0	1 low, 1 unclear
Inconsistency	Not serious	0	Difficult to determine due to the small number of studies, but no strong evidence of inconsistency
Indirectness	Not serious	0	Both studies looked at the effect of milk/dairy products on IGFBP-I levels
Imprecision	Serious	-1	Based on just 2 studies
Publication bias	Serious	-1	Although not clearly evident there is likely to be publication bias in this area of research
Factors increasing confidence			
Strength of association		0	No evidence of an association in either study
Dose-response		0	No evidence of a dose response
Confounders likely to minimise the effect		0	Unable to rule out confounding, may play a role
Final numerical rating of quality of evidence		1	
Statement of quality of evidence		There is currently a very low level of evidence linking milk with IGFBP-1 levels and this suggests no association.	

Supplementary Table 9 – GRADE assessment of studies of milk and IGFBP-2 levels

Quality assessment	**Rating**	**Adjustment to rating**	**Notes**
No of studies/starting rating	3 observational studies	2	Only 3 studies all observational
Factors decreasing confidence			
Limitations in study design (risk of bias)	Not serious	0	2 low, 1 unclear
Inconsistency	Not serious	0	Difficult to determine due to the small number of studies, but no strong evidence of inconsistency
Indirectness	Not serious	0	Both studies looked at the effect of milk/dairy products on IGFBP-2 levels
Imprecision	Serious	-1	Based on just 3 studies
Publication bias	Serious	-1	Although not clearly evident there is likely to be publication bias in this area of research
Factors increasing confidence			
Strength of association		0	Some evidence of a negative association in 2 studies but no evidence of an association in the 3rd
Dose-response		0	No evidence of a dose response
Confounders likely to minimise the effect		0	Unable to rule out confounding, may play a role
Final numerical rating of quality of evidence		1	
Statement of quality of evidence		There is currently a very low level of evidence linking milk with IGFBP-2 levels and this suggests a negative association.	

Supplementary Table 10 – GRADE assessment of studies of milk and IGFBP3 levels

Quality assessment	**Rating**	**Adjustment to rating**	**Notes**
No of studies/starting rating	13 studies, 2 of which are RCTs	2	A large number of observational studies with a smaller number of RCTs therefore start with score of 2
Factors decreasing confidence			
Limitations in study design (risk of bias)	Not serious	-1	Most studies have an unclear RoB
Inconsistency	Not serious	0	Studies seem to be consistent
Indirectness	Not serious	0	All studies looked at the effect of milk/dairy products on IGFBP-3 levels
Imprecision	Serious	0	Several studies, some with large sample size (>1000)
Publication bias	Serious	-1	Although not clearly evident there is likely to be publication bias in this area of research
Factors increasing confidence			
Strength of association		+1	Combined p-value is very low
Dose-response		0	Studies were not able to examine this robustly (RCTs based on a single dose/intervention, or food frequency questionnaires)
Confounders likely to minimise the effect		0	Unable to rule out confounding, may play a role
Final numerical rating of quality of evidence		1	
Statement of quality of evidence		There is currently a very low level of evidence linking milk and IGFBP-3 levels, and this suggests a negative association	

Supplementary table 11 – GRADE assessment of studies of IGF-I levels and prostate cancer risk

Quality assessment	**Rating**	**Adjustment to rating**	**Notes**
No of studies/starting rating	many observational studies	2	All observational
Factors decreasing confidence			
Limitations in study design (risk of bias)	Not serious	0	Moderate risk of bias
Inconsistency	Not serious	0	Studies show some effects in other directions, although prospective studies are mostly homogeneous
Indirectness	Not serious	0	All studies looked at circulating levels of IGF-I and their association with prostate cancer risk
Imprecision	Serious	0	Large number of studies some very large
Publication bias	Serious	0	No evidence of publication bias
Factors increasing confidence			
Strength of association		0	Fairly weak association
Dose-response		1	Some evidence of dose response
Confounders likely to minimise the effect		0	Unable to rule out confounding, may play a role
Final numerical rating of quality of evidence		3	
Statement of quality of evidence		There is currently a moderate level of evidence linking IGF-I levels to prostate cancer risk, and this suggests a positive association	

Supplementary table 12 – GRADE assessment of studies of IGF-II levels and prostate cancer risk

Quality assessment	**Rating**	**Adjustment to rating**	**Notes**
No of studies/starting rating	10 observational studies	2	All observational
Factors decreasing confidence			
Limitations in study design (risk of bias)	Not serious	0	Moderate risk of bias
Inconsistency	Not serious	-1	Studies show strong effects in opposite directions
Indirectness	Not serious	0	All studies looked at circulating levels of IGF-II and their association with prostate cancer risk
Imprecision	Serious	-1	Small number of studies some with conflicting results
Publication bias	Serious	0	No evidence of publication bias
Factors increasing confidence			
Strength of association		0	Fairly weak association
Dose-response		0	Possible dose response but inconsistency between studies
Confounders likely to minimise the effect		0	Unable to rule out confounding, may play a role
Final numerical rating of quality of evidence		1	
Statement of quality of evidence		There is currently a very low level of evidence linking IGF-II levels with prostate cancer and the evidence suggests a positive association	

Supplementary table 13 – GRADE assessment of studies of IGFBPI levels and prostate cancer risk

Quality assessment	**Rating**	**Adjustment to rating**	**Notes**
No of studies/starting rating	4 observational studies	2	All observational
Factors decreasing confidence			
Limitations in study design (risk of bias)	Not serious	0	Moderate risk of bias
Inconsistency	Not serious	-1	Studies show strong effects in opposite directions
Indirectness	Not serious	0	All studies looked at circulating levels of IGFBP-I and their association with prostate cancer risk
Imprecision	Serious	-1	Small number of studies overall wide confidence intervals
Publication bias	Serious	0	No evidence of publication bias
Factors increasing confidence			
Strength of association		0	Fairly weak association
Dose-response		0	Possible dose response but inconsistency between studies
Confounders likely to minimise the effect		0	Unable to rule out confounding, may play a role
Final numerical rating of quality of evidence		1	
Statement of quality of evidence		There is currently a very low level of evidence linking IGFBPI levels with prostate cancer risk and this suggests no association	

Supplementary table 14 – GRADE assessment of studies of IGFBP2 and prostate cancer risk

Quality assessment	**Rating**	**Adjustment to rating**	**Notes**
No of studies/starting rating	6 observational studies	2	All observational
Factors decreasing confidence			
Limitations in study design (risk of bias)	Not serious	0	Moderate risk of bias
Inconsistency	Not serious	-1	Studies show strong effects in opposite directions
Indirectness	Not serious	0	All studies looked at circulating levels of IGFBP-2 and their association with prostate cancer risk
Imprecision	Serious	-1	Small number of studies overall wide confidence intervals
Publication bias	Serious	0	No evidence of publication bias
Factors increasing confidence			
Strength of association		0	Fairly weak association
Dose-response		0	Possible dose response but inconsistency between studies
Confounders likely to minimise the effect		0	Unable to rule out confounding, may play a role
Final numerical rating of quality of evidence		1	
Statement of quality of evidence		There is currently a very low level of evidence linking IGFBP2 levels with prostate cancer.	

Supplementary table 15 – GRADE assessment of studies of IGFBP3 and prostate cancer risk

Quality assessment	**Rating**	**Adjustment to rating**	**Notes**
No of studies/starting rating	Many observational studies	2	All observational
Factors decreasing confidence			
Limitations in study design (risk of bias)	Not serious	0	Moderate risk of bias
Inconsistency	Not serious	0	Some studies show effects in opposite directions, prospective studies are largely homogeneous
Indirectness	Not serious	0	All studies looked at circulating levels of IGFBP-2 and their association with prostate cancer risk
Imprecision	Serious	0	Large number of studies some very large
Publication bias	Serious	0	No evidence of publication bias
Factors increasing confidence			
Strength of association		0	Fairly weak association
Dose-response		1	Some evidence of dose response
Confounders likely to minimise the effect		0	Unable to rule out confounding, may play a role
Final numerical rating of quality of evidence		3	
Statement of quality of evidence		There is currently a moderate level of evidence linking IGFBP3 levels with prostate cancer.	

Supplementary table 16 – GRADE assessment of IGFI and advanced prostate cancer risk

Quality assessment	**Rating**	**Adjustment to rating**	**Notes**
No of studies/starting rating	observational studies	2	All observational
Factors decreasing confidence			
Limitations in study design (risk of bias)	Not serious	0	Moderate risk of bias
Inconsistency	Not serious	-1	Some outliers and difference between prospective and retrospective studies
Indirectness	Not serious	0	All studies looked at circulating levels of IGF-I and their association with advanced prostate cancer risk
Imprecision	Serious	0	Overall estimate quite precise
Publication bias	Serious	0	No evidence of publication bias
Factors increasing confidence			
Strength of association		0	Fairly weak/no association
Dose-response		0	Possible dose response but inconsistency between studies
Confounders likely to minimise the effect		0	Unable to rule out confounding, may play a role
Final numerical rating of quality of evidence		1	
Statement of quality of evidence		There is currently a low level of evidence linking IGFI levels with advanced prostate cancer risk. This evidence suggests a positive association.	

Supplementary table 17 – GRADE assessment of IGFBP3 and advanced prostate cancer risk

Quality assessment	**Rating**	**Adjustment to rating**	**Notes**
No of studies/starting rating	observational studies	2	All observational
Factors decreasing confidence			
Limitations in study design (risk of bias)	Not serious	0	Moderate risk of bias
Inconsistency	Not serious	0	Some difference between prospective and retrospective studies
Indirectness	Not serious	0	All studies looked at circulating levels of IGFBP3 and their association with advanced prostate cancer risk
Imprecision	Serious	0	Overall estimate quite precise
Publication bias	Serious	0	No evidence of publication bias
Factors increasing confidence			
Strength of association		0	Fairly weak/no association
Dose-response		0	No evidence of dose response
Confounders likely to minimise the effect		0	Unable to rule out confounding, may play a role
Final numerical rating of quality of evidence		2	
Statement of quality of evidence		There is currently a low level of evidence linking IGFBP3 levels with advanced prostate cancer risk. Overall this evidence suggests no effect	

Supplementary table 18 – GRADE assessment of animal studies of the IGF pathway and prostate cancer risk

Quality assessment	**Rating**	**Adjustment to rating**	**Notes**
No of studies/starting rating	Experimental studies	4	All experimental
Factors decreasing confidence			
Limitations in study design (risk of bias)	Not serious	-1	All unclear risk of bias
Inconsistency	Not serious	0	Each study was very different so unable to assess this
Indirectness	Not serious	-1	Components of IGF pathway were knocked out or over expressed to very high levels, no comparable with normal distribution in humans, outcomes were tumour weight rather than incidence
Imprecision	Serious	-1	Small number of animals in each experiment, not able to combine results across studies
Publication bias	Serious	-1	Very likely to be publication bias
Factors increasing confidence			
Strength of association		0	1 study showed a strong association but not replicated
Dose-response		0	No evidence of dose response
Confounders likely to minimise the effect		0	Unable to rule out confounding, may play a role
Final numerical rating of quality of evidence		1	
Statement of quality of evidence		There is currently a very low level of evidence from animal studies linking the IGF pathway with prostate cancer risk.	

**To aid the adjustment to ratings, high quality evidence scores 4 as a starting rating, low quality evidence scores 2 and very low quality evidence scores 1. The initial starting rating is then adjusted as +1 or -1 based on factors that increase (+1) or decrease (-1) confidence in the quality of evidence. The minimum final numerical rating of quality of evidence is 1.*

Supplementary Box 1. Search strategies used to search MEDLINE and EMBASE (28th March 2014).

1. IGF1.tw
2. IGF-1.tw
3. IGFI.tw
4. IGF-I.tw
5. IGF1A.tw
6. IGF-IA.tw
7. IGF2.tw
8. IGF-2.tw
9. IGF-II.tw
10. IGFII.tw
11. IGF-IB.tw
12. IGF1B.tw
13. Insulin-like growth factor.tw
14. exp Somatomedins/
15. somatomedin*.tw
16. exp Insulin-Like Growth Factor Binding Proteins/
17. exp Receptors, Somatomedins/
18. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17
19. (prostat* adj3 (neoplas* or cancer or carcinoma or tumo?r)).tw
20. exp Prostatic Neoplasms/
21 19 or 20
22. exp Prostatic Intraepithelial neoplasia/
23. exp Neoplasm Metastasis/
24. exp Neoplasm Invasiveness/
25. 23 or 24
26. 21 and 25
27. 21 or 22 or 26
28. exp Dairy Products/
29. (dairy or milk or cheese* or butter or cream* or yog?urt).tw
30. 28 or 29
31. exp Pasteurization/
32. exp Dairying/
33. exp Food contamination/
34. (food* adj2 contaminat*).tw
35. 33 or 34
36. 30 and 35
37. exp Recombinant Proteins/
38. exp Growth Hormone/
39. exp Cattle/
40. 37 and 38 and 39
41. 30 or 31 or 32 or 36 or 40
42. 18 and 27
43. 42 not exp Therapeutic/
44. 43 not exp Review/
45. 18 and 41
46. 45 not exp Therapeutic/
47. 46 not exp Review/
48. 27 and 41
49. 48 not exp Therapeutic/
50. 49 not exp Review/
51. 18 and 27 and 41
52. 51 not exp Therapeutic/
53. 52 not exp Review/
54. 44 or 47 or 50 or 53

Note- for EMBASE use Therapy rather than Therapeutic MESH term

Supplementary Box 2. Search strategies used to search CINAHL (30th March 2014).

1. TI IGF1 or AB IGF1
2. TI IGF-1 or AB IGF-1
3. TI IGFI or AB IGFI
4. TI IGF-I or AB IGF-I
5. TI IGF1A or AB IGF1A
6. TI IGF-IA or AB IGF-IA
7. TI IGF2 or AB IGF2
8. TI IGF-2 or AB IGF-2
9. TI IGF-II or AB IGF-II
10. TI IGFII or AB IGFII
11. TI IGF-IB or AB IGF-IB
12. TI IGF1B or AB IGF1B
13. TI Insulin-like growth factor or AB Insulin-like growth factor
14. (MH "Somatomedins")
15. TI Insulin-like growth factor binding protein or AB Insulin-like growth factor binding protein
16. TI Somatomedins Receptors or AB Somatomedins Receptors
17. TI Insulin-like growth factor receptor or AB Insulin-like growth factor receptor
18. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17
19. (MH "Prostatic Neoplasms")
20. T1 Prostatic Intraepithelial neoplasia or AB Prostatic Intraepithelial neoplasia
21. T1 prostat* N3 (neoplas* or cancer or carcinoma or tumo?r) or AB prostat* N3 (neoplas* or cancer or carcinoma or tumo?r)
22. (MH "Neoplasm Metastasis+")
23. (MH "Neoplasm Invasiveness")
24. 22 or 23
25 19 or 21
26. 24 AND 25
27. 25 or 20 or 26
28. (MH "Dairy Products+")
29. T1 dairy or milk or cheese* or butter or cream* or yog?urt or AB dairy or milk or cheese* or butter or cream* or yog?urt
30. 28 or 29
31. (MH "Pasteurization")
32. TI Dairying or AB Dairying
33. (MH "Food Contamination+")
34. 30 AND 33
35. (MH "Recombinant Proteins+")
36. TI Growth Hormone or AB Growth Hormone
37. (MH "Cattle")
38. 35 AND 36 AND 37
39. 30 or 31 or 32 or 34 or 38
40. (MH "Therapeutics+")
41. (MH "Systematic Review")
42. (MH "Book Reviews")
43. (MH "Literature Review+")
44. 41 or 42 or 43
45. 18 AND 27
46. 45 not 40
47. 46 not 44
48. 18 AND 39
49. 48 not 40
50. 49 not 44
51. 27 AND 39
52. 51 not 40
53. 52 not 44
54. 18 AND 27 AND 39
55. 54 not 40
56. 55 not 44
57. 47 or 50 or 53 or 56

Supplementary Box 2. Search strategies used to search BIOSIS (31st March 2014).

1. Topic=(IGF1 or IGF-1 or IGFI or IGF-I or IGF1A or IGF-IA or IGF2 or IGF-2 or IGF-II or IGFII or IGF-IB or IGF1B)
2. Topic=(Insulin-like growth factor)
3. Topic=(Somatomedins)
4. Topic=(Insulin-like growth factor binding proteins)
5. Topic=(Somatomedins Receptors)
6. Topic=(Insulin-like growth factor receptor)
7. 6 OR 5 OR 4 OR 3 OR 2 OR 1
8. Topic=(prostat* neoplas* or prostat* cancer or prostat* carcinoma or prostat* tumo$r)
9. Topic=(prostatic intraepithelial neoplasia)
10. 9 OR 8
11. Topic=(Neoplasm Metastasis or Neoplasm Invasiveness)
12. 11 AND 8
13. 12 OR 10
14. Topic=(Dairy Products)
15. Topic=(dairy or milk or cheese* or butter or cream* or yog$urt)
16. Topic=(pasteurization or dairying)
17. Topic=(Food contamination)
18. 17 AND 14
19. Topic=(Recombinant Proteins)
20. Topic=(Growth Hormone)
21. Topic=(Cattle)
34. 33 AND 32 AND 31
35. 34 OR 30 OR 28 OR 27 OR 26
22. 21 AND 20 AND 19
23. 22 OR 18 OR 16 OR 15 OR 14
24. Topic=(Therapeutics)
25. Topic=(Review)
26. 13 AND 7
27. 26 NOT 24
28. 27 NOT 25
29. 23 AND 7
30. 29 NOT 24
31. 30 NOT 25
32. 23 AND 13
33. 32 NOT 24
34. 33 NOT 25
35. 23 AND 13 AND 7
36. 35 NOT 24
37. 36 NOT 25
38. 37 OR 34 OR 31 OR 28

Supplementary Box 4. Risk of bias protocol.

RoB Categories	Specific questions used to answer RoB categories
Milk-IGF (human): case/control	
Confounding	Did the authors identify all possible confounding factors? Were these taken into account in the study design and/or analysis? Was there a large difference between the characteristics of cases and controls and if so, were they adjusted for?
Selection of participants	Were they selected in an acceptable way? Were they part of a defined population?
Missing data	Was the follow-up long enough for the outcome to occur? Was the follow-up complete enough? Were details given of those lost to follow up? E.g., was there a difference in those lost to follow-up compared to those included in the study?
Measurement of outcome	Did the authors use objective measurements? Were they validated? Was it accurately measured?
Measurement of exposure *(Focus on information recall from participants)*	Did the authors use objective measurements? Were they validated? Were all subjects classified into exposure groups using the same procedure? Was it accurately measured? Did this involve information recall from the participants?
Selection of reported results	Was the full protocol available? Were all aims of the study reported? Was the study free of selective reporting?
Milk-IGF (human): RCT	
Sequence generation	Was the way in which participants were selected at random acceptable?
Allocation concealment	*Not applicable to this analysis as IGF levels cannot be directly influenced by the participants knowledge of the intervention they are participating in.*
Blinding of participants and personnel	*Not applicable to this analysis as IGF levels cannot be directly influenced by the participants knowledge of the intervention they are participating in.*
Blinding of outcome assessors	Were the assessors blinded to the analysis they were undertaking?
Incomplete data	Was the follow-up long enough for the outcome to occur? Was the follow-up complete enough? Were details given of those lost to follow up? E.g., was there a difference in those lost to follow-up compared to those included in the study?
Selective reporting	Was the full protocol available? Were all aims of the study reported? Was the study free of selective reporting?
IGF-PCa (human): all	
Confounding *(IGF level studies focused on age & ethnicity; genetics studies focused on age, disease status of controls & ethnicity)*	Did the authors identify all possible confounding factors? Were these taken into account in the study design and/or analysis? Was there a large difference between the characteristics of cases and controls and if so, were they adjusted for?
Selection of participants	Were they selected in an acceptable way? Were they part of a defined population?

Missing data (cohorts only)	Was the follow-up long enough for PCa to occur? Was the follow-up complete enough? Were details given of those lost to follow up? Was there a difference in those lost to follow-up compared to those included in the study?
Measurement of outcome (cohorts only)	Did the authors use objective measurements? Were they validated? Was it accurately measured?
Measurement of exposure	Did the authors use objective measurements? Were they validated? Were all subjects classified into exposure groups using the same procedure? Was it accurately measured?
Selection of reported results	Was the full protocol available? Were all aims of the study reported? Was the study free of selective reporting?
IGF-PCa (animal): all	
Confounding	Did the authors identify all possible confounding factors? Were these taken into account in the study design and/or analysis? Was there a large difference between the characteristics of cases and controls and if so, were they adjusted for?
Departures from intended observations	Were the original aims of the study met? Did the results answer the original study question?
Random housing of animals	Were animals housed and kept in the same way to minimise environmental factors?
Missing data	Was the follow-up long enough for the outcome to occur? Was the follow-up complete enough? Were details given of those lost to follow up? E.g., was there a difference in those lost to follow-up compared to those included in the study?
Measurement of outcome	Did the authors use objective measurements? Were they validated? Was it accurately measured?
Random outcome assessment of animals	Were samples consistently taken from experimental and control groups at the same time?
Selection of reported results	Was the full protocol available? Were all aims of the study reported? Was the study free of selective reporting?